

## CLAIMS

1. An analytical kit comprising the reagent A, reagent B and analytical device specified below in combination, wherein the reagent A and B may be contained in one and the same system or may occur each independently:

i) An analytical device comprising a passage allowing a liquid to flow through the same as formed by bonding together a first member having a groove, 1  $\mu\text{m}$  to 5 mm width and 1  $\mu\text{m}$  to 750  $\mu\text{m}$  depth in cross-section, and a second member capable of covering the groove, together with a first nucleic acid (N1) having an arbitrary base sequence as immobilized in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together;

ii) A reagent A containing a conjugate (N2-L1) composed of a second nucleic acid (N2) having a sequence at least complementary to the base sequence of the first nucleic acid (N1) immobilized in the capturing zone of the analytical device and a first ligand (L1) capable of specifically binding to a biological substance (O) to be assayed;

iii) A reagent B containing a conjugate (L2-M) resulting from binding of a marker (M) to a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed.

2. An analytical kit comprising the reagent A, reagent B', reagent C and analytical device specified below in combination, wherein two or more of the reagents A, B' and C may be contained

in one and the same system or the reagents may occur each independently:

- i) An analytical device comprising a passage allowing a liquid to flow through the same as formed by bonding together a first member having a groove, 1  $\mu\text{m}$  to 5 mm width and 1  $\mu\text{m}$  to 750  $\mu\text{m}$  depth in cross-section, and a second member capable of covering the groove, together with a first nucleic acid (N1) having an arbitrary base sequence as immobilized in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together;
- ii) A reagent A containing a conjugate (N2-L1) composed of a second nucleic acid (N2) having a sequence at least complementary to the base sequence of the first nucleic acid (N1) immobilized in the capturing zone of the analytical device and a first ligand (L1) capable of specifically binding to a biological substance (O) to be assayed;
- iii) A reagent B' containing a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed; and
- iv) A reagent C containing a conjugate (L3-M) composed of a third ligand (L3) capable of specifically binding to the second ligand (L2) and a marker (M).

3. An analytical kit comprising the reagent A and analytical device specified below in combination and containing no marker:

- i) An analytical device comprising a passage allowing a liquid to flow through the same as formed by bonding together a first

member having a groove, 1  $\mu\text{m}$  to 5 mm width and 1  $\mu\text{m}$  to 750  $\mu\text{m}$  depth in cross-section, and a second member capable of covering the groove, together with a first nucleic acid (N1) having an arbitrary base sequence as immobilized in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together; and

ii) A reagent A containing a conjugate (N2-L1) composed of a second nucleic acid (N2) having a sequence at least complementary to the base sequence of the first nucleic acid (N1) immobilized in the capturing zone of the analytical device and a first ligand (L1) capable of specifically binding to a biological substance (O) to be assayed.

4. An analytical kit comprising the reagent B and analytical device specified below in combination:

i) An analytical device comprising a passage allowing a liquid to flow through the same as formed by bonding together a first member having a groove, 1  $\mu\text{m}$  to 5 mm width and 1  $\mu\text{m}$  to 750  $\mu\text{m}$  depth in cross-section, and a second member capable of covering the groove, together with a first nucleic acid (N1) having an arbitrary base sequence as immobilized in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together, and further together with a conjugate (N2-L1) composed of a first ligand (L1) capable of specifically binding to a biological substance (O) to be assayed and a second nucleic acid (N2) having

a base sequence at least complementary to the immobilized first nucleic acid (N1) as formed and immobilized in the capturing zone by specific binding between the first nucleic acid (N1) and second nucleic acid (N2); and

ii) A reagent B containing a conjugate (L2-M) resulting from binding between a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed and a marker (M).

5. An analytical kit comprising the reagent A, reagent B', reagent C and analytical device specified below in combination, wherein two or more of the reagents A, B' and C may be contained in one and the same system or the reagents may occur each independently:

i) An analytical device comprising a passage allowing a liquid to flow through the same as formed by bonding together a first member having a groove, 1  $\mu$ m to 5 mm width and 1  $\mu$ m to 750  $\mu$ m depth in cross-section, and a second member capable of covering the groove, together with a first nucleic acid (N1) having an arbitrary base sequence as immobilized in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together, and further together with a conjugate (N2-L1) composed of a first ligand (L1) capable of specifically binding to a biological substance (O) to be assayed and a second nucleic acid (N2) having a base sequence at least complementary to the immobilized first nucleic acid (N1) as formed and immobilized in the capturing

zone by specific binding between the first nucleic acid (N1) and second nucleic acid (N2); and

ii) A reagent B' containing a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed; and

iii) A reagent C containing a conjugate (L3-M) composed of a third ligand (L3) capable of specifically binding to the second ligand (L2) and a marker (M).

6. An analytical kit comprising the reagent A, reagent B and analytical device specified below in combination, wherein the reagent A and B may be contained in one and the same system or may occur each independently:

i) An analytical device comprising a passage allowing a liquid to flow through the same as formed by bonding together a first member having a groove, 1  $\mu$ m to 5 mm width and 1  $\mu$ m to 750  $\mu$ m depth in cross-section, and a second member capable of covering the groove, together with a plurality of first nucleic acid species (N1g: g being an integer) each having an arbitrary base sequence as immobilized each independently, from species to species, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together;

ii) A reagent A containing a plurality of conjugate species (N2h-L1i: h and i each independently being an integer) each composed of one of a plurality of second nucleic acid species (N2h: h being an integer) each having a sequence at least

complementary to the base sequence of the corresponding one among the plurality of first nucleic acid species (Nlg: g being an integer) immobilized in the capturing zone and one of a plurality of first ligand species (Lli: i being an integer) which is capable of specifically binding to the corresponding one among one or more biological substance species (Ok: k being an integer) to be assayed; and

iii) A reagent B containing conjugate species (L2j-Ml: j and l each independently being an integer) resulting from binding between one or more second ligand species (L2j: j being an integer) capable of specifically binding to the corresponding one or more biological substance species (Ok: k being an integer) to be assayed and one or more marker species (Ml: l being an integer).

7. An analytical kit comprising the reagent A, reagent B', reagent C and analytical device specified below in combination, wherein two or more of the reagents A, B' and C may be contained in one and the same system or the reagents may occur each independently:

i) An analytical device comprising a passage allowing a liquid to flow through the same as formed by bonding together a first member having a groove, 1  $\mu$ m to 5 mm width and 1  $\mu$ m to 750  $\mu$ m depth in cross-section, and a second member capable of covering the groove, together with a plurality of first nucleic acid species (Nlg: g being an integer) each having an arbitrary base sequence as immobilized each independently, from species to species, in a capturing zone provided in the passage on the first

member and/or second member prior to bonding the first member and second member together;

ii) A reagent A containing a plurality of conjugate species ( $N2h-L1i$ :  $h$  and  $i$  each independently being an integer) each composed of one of a plurality of second nucleic acid species ( $N2h$ :  $h$  being an integer) each having a sequence at least complementary to the base sequence of the corresponding one among the plurality of first nucleic acid species ( $N1g$ :  $g$  being an integer) immobilized in the capturing zone and one of a plurality of first ligand species ( $L1i$ :  $i$  being an integer) which is capable of specifically binding to the corresponding one among one or more biological substance species ( $Ok$ :  $k$  being an integer) to be assayed;

iii) A reagent B' containing one or more second ligand species ( $L2j$ :  $j$  being an integer) each capable of specifically binding to the corresponding one among the one or more biological substance species ( $Ok$ :  $k$  being an integer) to be assayed; and

iv) A reagent C containing conjugate species ( $L3m-M1$ :  $m$  and  $l$  each independently being an integer) composed of one or more third ligand species ( $L3m$ :  $m$  being an integer) capable of specifically binding to the corresponding one among the one or more second ligand species ( $L2j$ :  $j$  being an integer) and one or more marker species ( $M1$ :  $l$  being an integer).

8. An analytical kit comprising the reagent A and analytical device specified below in combination and containing no marker:

i) An analytical device comprising a passage allowing a liquid

to flow through the same as formed by bonding together a first member having a groove, 1  $\mu\text{m}$  to 5 mm width and 1  $\mu\text{m}$  to 750  $\mu\text{m}$  depth in cross-section, and a second member capable of covering the groove, together with a plurality of first nucleic acid species ( $\text{N}1\text{g}$ : g being an integer) each having an arbitrary base sequence as immobilized each independently, from species to species, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together;

ii) A reagent A containing a plurality of conjugate species ( $\text{N}2\text{h-L}1\text{i}$ : h and i each independently being an integer) each composed of one of a plurality of second nucleic acid species ( $\text{N}2\text{h}$ : h being an integer) each having a sequence at least complementary to the base sequence of the corresponding one among the plurality of first nucleic acid species ( $\text{N}1\text{g}$ : g being an integer) immobilized each independently, from species to species, in the capturing zone of the analytical device and one of a plurality of first ligand species ( $\text{L}1\text{i}$ : i being an integer) which is capable of specifically binding to the corresponding one among one or more biological substance species ( $\text{O}k$ : k being an integer) to be assayed.

9. An analytical kit comprising the reagent B and analytical device specified below in combination:

i) An analytical device comprising a passage allowing a liquid to flow through the same as formed by bonding together a first member having a groove, 1  $\mu\text{m}$  to 5 mm width and 1  $\mu\text{m}$  to 750  $\mu\text{m}$



depth in cross-section, and a second member capable of covering the groove, together with a plurality of first nucleic acid species ( $N1g$ :  $g$  being an integer) each having an arbitrary base sequence as immobilized each independently, from species to species, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together, and further together with conjugate species ( $N2h-L1i$ :  $h$  and  $i$  each independently being an integer) each composed of one of a plurality of first ligand species ( $L1i$ :  $i$  being an integer), which is capable of specifically binding to the corresponding one among one or more biological substance species ( $Ok$ :  $k$  being an integer) to be assayed, and one of a plurality of second nucleic acid species ( $N2h$ :  $h$  being an integer), which has a base sequence at least complementary to the corresponding one among the immobilized first nucleic acid species ( $N1g$ :  $g$  being an integer), as formed and immobilized in the capturing zone by specific binding between the first nucleic acid species and second nucleic acid species; and

ii) A reagent B containing conjugate species ( $L2j-M1$ :  $j$  and  $l$  each independently being an integer) resulting from binding between one or more second ligand species ( $L2j$ :  $j$  being an integer) respectively capable of specifically binding to the corresponding one or more biological substance species to be assayed and one or more marker species ( $M1$ :  $l$  being an integer).

10. An analytical kit comprising the reagent B', reagent C and analytical device specified below in combination:

- i) An analytical device comprising a passage allowing a liquid to flow through the same as formed by bonding together a first member having a groove, 1  $\mu\text{m}$  to 5 mm width and 1  $\mu\text{m}$  to 750  $\mu\text{m}$  depth in cross-section, and a second member capable of covering the groove, together with a plurality of first nucleic acid species ( $\text{N1g}$ : g being an integer) each having an arbitrary base sequence as immobilized each independently, from species to species, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together, and further together with conjugate species ( $\text{N2h-L1i}$ : h and i each independently being an integer) each composed of one of a plurality of first ligand species ( $\text{L1i}$ : i being an integer), which is capable of specifically binding to the corresponding one among one or more biological substance species ( $\text{Ok}$ : k being an integer) to be assayed, and one of a plurality of second nucleic acid species ( $\text{N2h}$ : h being an integer), which has a base sequence at least complementary to the corresponding one among the immobilized first nucleic acid species ( $\text{N1g}$ : g being an integer), as formed and each independently immobilized in the capturing zone by specific binding between the first nucleic acid species and second nucleic acid species; and
- ii) A reagent B' containing one or more second ligand species ( $\text{L2j}$ : j being an integer) capable of specifically binding to the corresponding one or more biological substance species ( $\text{Ok}$ : k being an integer) to be assayed;
- iii) A reagent C containing conjugate species ( $\text{L3m-M1}$ : m and

l each independently being an integer) derived from one or more third ligand species (L3m: m being an integer) capable of specifically binding to the corresponding one or more second ligand species (L2j: j being an integer) and one or more marker species (Ml: l being an integer).

11. An analytical kit according to any of Claims 1 to 10, wherein the biological substance(s), first ligand(s) (L1 or L1i: i being an integer), second ligand(s) (L2 or L2j: j being an integer) and/or third ligand(s) (L3 or L3m: m being an integer) is/are selected from among immunological substances, receptors, receptor-binding substances, sugars, glycoproteins, glycolipids, lectins and nucleic acids.

12. An analytical kit according to Claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, wherein the first ligand or ligands (L1 or L1i: i being an integer) and/or second ligand or ligands (L2 or L2j: j being an integer) are different in reactivity.

13. An analytical kit according to Claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, wherein the first ligand or ligands (L1 or L1i: i being an integer) and/or second ligand or ligands (L2 or L2j: j being an integer) are identical in reactivity.

14. An analytical kit according to any of Claims 1 to 10, wherein the marker or markers (M or Ml: l being an integer) each is selected from among enzymes, colloidal metals, latexes, nucleic acids,

luminescent substances, fluorescent substances, intercalators, biotin, avidin and streptavidin.

15. An analytical kit according to any of Claims 1 to 10, wherein the first member or second member material is selected from among glass, polydimethylsiloxane, ceramics, acrylonitrile-butadiene rubber-styrene resins, acrylonitrile-ethylenepropylene rubber-styrene resins, acrylonitrile-styrene resins, methacrylic-styrene resins, polyamide nylon resins, polybutylene terephthalate resins, polycarbonate resins, polyethylene resins, polyethylene resins, polyethylene terephthalate polyester resins, polyimide resins, methacrylic resins, polyacetal resins, polypropylene resins, polyphenylene ether resins, polyphenylene sulfide resins, polystyrene resins, thermoplastic elastomer resins, alloys, liquid crystal polymer resins, cycloolefin resins, thermoplastic resins, epoxy resins, phenol resins, unsaturated polyester resins, diallyl phthalate resins, cyclic olefin copolymers and, further, materials derived from these materials by surface modification.

16. An analytical kit according to any of Claims 1 to 10, wherein the first member and second member are made of the same material.

17. An analytical kit according to any of Claims 1 to 10, wherein the material of the first member and the material of the second member are different from each other.

18. An analytical device comprising a passage allowing a liquid to flow through the same as formed by bonding together a first member having a groove, 1  $\mu\text{m}$  to 5 mm width and 1  $\mu\text{m}$  to 750  $\mu\text{m}$  depth in cross-section, and a second member capable of covering the groove, together with a first nucleic acid (N1) having an arbitrary base sequence as immobilized in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together, said device further comprising a conjugate (N2-L1) composed of a first ligand (L1) capable of specifically binding to a biological substance (O) to be assayed and a second nucleic acid (N2) having a base sequence at least complementary to the immobilized first nucleic acid (N1) as formed and immobilized in the capturing zone by specific binding between the first nucleic acid (N1) and second nucleic acid (N2).

19. An analytical device comprising a passage allowing a liquid to flow through the same as formed by bonding together a first member having a groove, 1  $\mu\text{m}$  to 5 mm width and 1  $\mu\text{m}$  to 750  $\mu\text{m}$  depth in cross-section, and a second member capable of covering the groove, together with a plurality of first nucleic acid species (N1g: g being an integer) each having an arbitrary base sequence as immobilized each independently, from species to species, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together, said device further comprising

conjugate species ( $N2h-L1i$ :  $h$  and  $i$  each independently being an integer) each composed of one of a plurality of first ligand species ( $L1i$ :  $i$  being an integer), which is capable of specifically binding to the corresponding one among one or more biological substance species ( $Ok$ :  $k$  being an integer) to be assayed, and one of a plurality of second nucleic acid species ( $N2h$ :  $h$  being an integer), which has a base sequence at least complementary to the corresponding one among the immobilized first nucleic acid species ( $N1g$ :  $g$  being an integer), as formed and immobilized each independently, from species to species, in the capturing zone by specific binding between the first nucleic acid species and second nucleic acid species.

20. An analytical device according to Claim 18 or 19, wherein the biological substance or substances ( $O$  or  $Ok$ :  $k$  being an integer) and/or first ligand or ligands ( $L1$  or  $L1i$ :  $i$  being an integer) are selected from among immunological substances, receptors and nucleic acids.

21. An analytical device according to Claim 18 or 19, wherein the first member or second member material is selected from among glass, polydimethylsiloxane, ceramics, acrylonitrile-butadiene rubber-styrene resins, acrylonitrile-ethylenepropylene rubber-styrene resins, acrylonitrile-styrene resins, methacrylic-styrene resins, polyamide nylon resins, polybutylene terephthalate resins, polycarbonate resins, polyethylene resins, polyethylene resins,

polyethylene terephthalate polyester resins, polyimide resins, methacrylic resins, polyacetal resins, polypropylene resins, polyphenylene ether resins, polyphenylene sulfide resins, polystyrene resins, thermoplastic elastomer resins, alloys, liquid crystal polymer resins, cycloolefin resins, thermoplastic resins, epoxy resins, phenol resins, unsaturated polyester resins, diallyl phthalate resins, cyclic olefin copolymers and, further, materials derived from these materials by surface modification.

22. An analytical device according to Claim 18 or 19, wherein the first member and second member are made of the same material.

23. An analytical device according to Claim 18 or 19, wherein the material of the first member and the material of the second member are different from each other.

24. An analytical method comprising the following elements i) to iv):

i) Preparing an analytical device comprising a passage allowing a liquid to flow through the same as formed by bonding together a first member having a groove, 1  $\mu\text{m}$  to 5 mm width and 1  $\mu\text{m}$  to 750  $\mu\text{m}$  depth in cross-section, and a second member capable of covering the groove, together with a first nucleic acid (N1) having an arbitrary base sequence as immobilized in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member

together;

ii) Preparing a reagent A containing a conjugate (N2-L1) resulting from binding of a first ligand (L1) capable of specifically binding to a biological substance to be assayed to a second nucleic acid (N2) having a sequence at least complementary to the base sequence of the first nucleic acid (N1);

iii) Introducing a liquid sample suspected of the occurrence therein of the biological substance to be assayed and the reagent A, either after preliminary mixing thereof for conjugate formation or while allowing conjugate formation, into the passage in the analytical device for immobilizing the resulting conjugate within the passage;

iv) Assaying the immobilized conjugate.

25. An analytical method comprising the following elements

i) to iv):

i) Preparing an analytical device comprising a passage allowing a liquid to flow through the same as formed by bonding together a first member having a groove, 1  $\mu\text{m}$  to 5 mm width and 1  $\mu\text{m}$  to 750  $\mu\text{m}$  depth in cross-section, and a second member capable of covering the groove, together with a first nucleic acid (N1) having an arbitrary base sequence as immobilized in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together;

ii) Preparing a reagent A containing a conjugate (N2-L1)



resulting from binding of a first ligand (L1) capable of specifically binding to a biological substance to be assayed to a second nucleic acid (N2) having a sequence at least complementary to the base sequence of the first nucleic acid (N1);

- iii) Introducing a liquid sample suspected of the occurrence therein of the biological substance to be assayed and the reagent A individually, without preliminary mixing together, into the passage in the analytical device for immobilizing the resulting conjugate within the passage;
- iv) Assaying the immobilized conjugate.

26. An analytical method comprising the following elements i) to iv):

- i) Preparing an analytical device comprising a passage allowing a liquid to flow through the same as formed by bonding together a first member having a groove, 1  $\mu\text{m}$  to 5 mm width and 1  $\mu\text{m}$  to 750  $\mu\text{m}$  depth in cross-section, and a second member capable of covering the groove, together with a plurality of first nucleic acid species (N1g: g being an integer) each having an arbitrary base sequence as immobilized each independently, from species to species, in a capturing zone provided in the passage on the first member and/or second member prior to bonding the first member and second member together;
- ii) Preparing a reagent A containing a plurality of conjugate species (N2h-L1i: h and i each independently being an integer) each resulting from binding of one of a plurality of first ligand

species ( $L_{li}$ :  $i$  being an integer), which is capable of specifically binding to the corresponding one among one or more biological substance species ( $O_k$ :  $k$  being an integer) to be assayed, to one of a plurality of second nucleic acid species ( $N_{2h}$ :  $h$  being an integer) each having a sequence at least complementary to the base sequence of the corresponding one among the plurality of first nucleic acid species ( $N_{1g}$ :  $g$  being an integer);

iii) Introducing a liquid sample suspected of the occurrence therein of one or more biological substance species ( $O_k$ :  $k$  being an integer) to be assayed and the reagent A, either after preliminary mixing thereof for conjugate formation or while allowing conjugate formation, into the passage in the analytical device for immobilizing the resulting one or more conjugates within the passage;

iv) Assaying the immobilized conjugate(s).

27. An analytical method comprising the following elements i) to iv):

i) Preparing an analytical device comprising a passage allowing a liquid to flow through the same as formed by bonding together a first member having a groove, 1  $\mu\text{m}$  to 5 mm width and 1  $\mu\text{m}$  to 750  $\mu\text{m}$  depth in cross-section, and a second member capable of covering the groove, together with a plurality of first nucleic acid species ( $N_{1g}$ :  $g$  being an integer) each having an arbitrary base sequence as immobilized each independently, from species to species, in a capturing zone provided in the passage on the

first member and/or second member prior to bonding the first member and second member together;

ii) Preparing a reagent A containing a plurality of conjugate species ( $N2h-L1i$ :  $h$  and  $i$  each independently being an integer) each resulting from binding of one of a plurality of first ligand species ( $L1i$ :  $i$  being an integer), which is capable of specifically binding to the corresponding one among one or more biological substance species ( $Ok$ :  $k$  being an integer) to be assayed, to one of a plurality of second nucleic acid species ( $N2h$ :  $h$  being an integer) each having a sequence at least complementary to the base sequence of the corresponding one among the plurality of first nucleic acid species ( $N1g$ :  $g$  being an integer);

iii) Introducing a liquid sample suspected of the occurrence therein of one or more biological substances ( $Ok$ :  $k$  being an integer) to be assayed and the reagent A individually into the passage in the analytical device for immobilizing the resulting one or more conjugates within the passage;

iv) Assaying the immobilized conjugate(s).

28. An analytical method comprising the following elements

i) to iv):

i) Using the analytical kit according to Claim 1;

ii) Introducing two or more of the materials a, b and c given below, either after preliminary mixing thereof for conjugate formation or while allowing conjugate formation, into the passage in the analytical device contained in the analytical kit,

followed by introduction of the remaining material, if any, into the passage:

a. A liquid sample suspected of the occurrence therein of a biological substance (O) to be assayed,

b. A reagent A containing a conjugate (N2-L1) composed of a second nucleic acid (N2) having a base sequence at least complementary to the base sequence of the first nucleic acid (N1) immobilized in the capturing zone and a first ligand (L1) capable of specifically binding to the biological substance (O) to be assayed,

c. A reagent B containing a conjugate (L2-M) resulting from direct binding of a marker to a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed;

iii) Allowing the formation of an immobilized conjugate (N1-N2-L1-O-L2-M) by specific binding between the first nucleic acid (N1) immobilized in the capturing zone in the analytical device and the second nucleic acid (N2), specific binding between the first ligand (L1) and biological substance (O) and specific binding between the second ligand (L2) and biological substance (O);

iv) Assaying the biological substance (O) by assaying the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-L2-M).

29. An analytical method comprising the following elements

i) to iv):

- i) Using the analytical kit according to Claim 1;
- ii) Introducing the following materials a, b and c given below individually, without mixing together, into the passage in the analytical device contained in the analytical kit:
  - a. A liquid sample suspected of the occurrence therein of a biological substance (O) to be assayed,
  - b. A reagent A containing a conjugate (N2-L1) composed of a second nucleic acid (N2) having a base sequence at least complementary to the base sequence of the first nucleic acid (N1) immobilized in the capturing zone and a first ligand (L1) capable of specifically binding to the biological substance (O) to be assayed,
  - c. A reagent B containing a conjugate (L2-M) resulting from direct binding of a marker (M) to a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed;
- iii) Allowing the formation of an immobilized conjugate (N1-N2-L1-O-L2-M) by specific binding between the first nucleic acid (N1) immobilized in the capturing zone in the analytical device and the second nucleic acid (N2), specific binding between the first ligand (L1) and biological substance (O) and specific binding between the second ligand (L2) and biological substance (O);
- iv) Assaying the biological substance (O) by assaying the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-L2-M).

30. An analytical method comprising the following elements i) to iv):

i) Using the analytical kit according to Claim 2;  
ii) Introducing two or more of the materials a, b, c and d given below, either after preliminary mixing thereof for conjugate formation or while allowing conjugate formation, into the passage in the analytical device contained in the analytical kit, followed by introduction of the remaining material or materials, if any, into the passage:

a. A liquid sample suspected of the occurrence therein of a biological substance (O) to be assayed,

b. A reagent A containing a conjugate (N2-L1) composed of a second nucleic acid (N2) having a base sequence at least complementary to the base sequence of the first nucleic acid (N1) immobilized in the capturing zone and a first ligand (L1) capable of specifically binding to the biological substance (O) to be assayed,

c. A reagent B' containing a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed, and

d. A reagent C containing a conjugate (L3-M) composed of a third ligand (L3) capable of specifically binding to the second ligand (L2) and a marker (M);

iii) Allowing the formation of an immobilized conjugate (N1-N2-L1-O-L2-L3-M) by specific binding between the first nucleic acid (N1) immobilized in the capturing zone in the analytical device and the second nucleic acid (N2), specific

binding between the first ligand (L1) and biological substance (O), specific binding between the second ligand (L2) and biological substance (O) and specific binding between the second ligand (L2) and third ligand (L3);

iv) Assaying the biological substance (O) by assaying the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-L2-L3-M).

31. An analytical method comprising the following elements i) to iv):

i) Using the analytical kit according to Claim 2;  
ii) Introducing the following materials a, b, c and d individually, without any mixing, into the passage in the analytical device contained in the analytical kit:

a. A liquid sample suspected of the occurrence therein of a biological substance (O) to be assayed,

b. A reagent A containing a conjugate (N2-L1) composed of a second nucleic acid (N2) having a base sequence at least complementary to the base sequence of the first nucleic acid (N1) immobilized in the capturing zone and a first ligand (L1) capable of specifically binding to the biological substance (O) to be assayed,

c. A reagent B' containing a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed, and

d. A reagent C containing a conjugate (L3-M) composed of a third ligand (L3) capable of specifically binding to the

second ligand (L2) and a marker (M);

iii) Allowing the formation of an immobilized conjugate (N1-N2-L1-O-L2-L3-M) by specific binding between the first nucleic acid (N1) immobilized in the capturing zone in the analytical device and the second nucleic acid (N2), specific binding between the first ligand (L1) and biological substance (O), specific binding between the second ligand (L2) and biological substance (O) and specific binding between the second ligand (L2) and third ligand (L3);

iv) Assaying the biological substance (O) by assaying the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-L2-L3-M).

32. An analytical method comprising the following elements i) to v):

i) Using the analytical kit according to Claim 3;

ii) Preparing a marker-carrying biological substance (O-M) in advance from a liquid sample suspected of the occurrence therein of a biological substance (O) to be assayed by introduction of a marker (M) into that substance;

iii) Introducing a reagent A containing a conjugate (N2-L1) composed of a second nucleic acid (N2) having a base sequence at least complementary to the base sequence of the first nucleic acid (N1) immobilized in the capturing zone and a first ligand (L1) capable of specifically binding to the biological substance (O) to be assayed and the marker-carrying biological substance (O-M), either after preliminary mixing up for conjugate formation



or while allowing conjugate formation, into the passage in the analytical device contained in the analytical kit;

iv) Allowing the formation of an immobilized conjugate (N1-N2-L1-O-M) by specific binding between the first nucleic acid (N1) immobilized in the capturing zone in the analytical device and the second nucleic acid (N2);

v) Assaying the biological substance (O) by assaying the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-M).

33. An analytical method comprising the following elements i) to v):

i) Using the analytical kit according to Claim 3;

ii) Preparing a marker-carrying biological substance (O-M) in advance from a liquid sample suspected of the occurrence therein of a biological substance (O) to be assayed by introduction of a marker (M) into that substance;

iii) Introducing a reagent A containing a conjugate (N2-L1) composed of a second nucleic acid (N2) having a base sequence at least complementary to the base sequence of the first nucleic acid (N1) immobilized in the capturing zone and a first ligand (L1) capable of specifically binding to the biological substance (O) to be assayed and the marker-carrying biological substance (O-M) individually, without mixing together, into the passage in the analytical device contained in the analytical kit;

iv) Allowing the formation of an immobilized conjugate (N1-N2-L1-O-M) by specific binding between the first nucleic

acid (N1) immobilized in the capturing zone in the analytical device and the second nucleic acid (N2);

v) Assaying the biological substance (O) by assaying the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-M).

34. An analytical method comprising the following elements i) to iv):

i) Using the analytical kit according to Claim 4;  
ii) Introducing the materials a and b given below, either after preliminary mixing up for conjugate formation or while allowing conjugate formation, into the passage in the analytical device contained in the analytical kit:

a. A liquid sample suspected of the occurrence of a biological substance (O) to be assayed,

b. A reagent containing a conjugate (L2-M) resulting from direct binding between a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed and a marker (M):

iii) Allowing the formation of an immobilized conjugate (N1-N2-L1-O-L2-M) by specific binding between the first ligand (L1) in the conjugate (N1-N2-L1) immobilized in the capturing zone in the analytical device and the biological substance (O) and by specific binding between the second ligand (L2) in the conjugate (L2-M) and the biological substance (O);

iv) Assaying the biological substance (O) by assaying the marker (M) contained in the immobilized conjugate

(N1-N2-L1-O-L2-M) .

35. An analytical method comprising the following elements i) to iv):

i) Using the analytical kit according to Claim 4;  
ii) Introducing the following materials a and b individually, without mixing together, into the passage in the analytical device contained in the analytical kit:

a. A liquid sample suspected of the occurrence therein of a biological substance (O) to be assayed,

b. A reagent containing a conjugate (L2-M) resulting from direct binding between a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed and a marker (M);

iii) Allowing the formation of an immobilized conjugate (N1-N2-L1-O-L2-M) by specific binding between the first ligand (L1) in the conjugate (N1-N2-L1) immobilized in the capturing zone in the analytical device and the biological substance (O) and by specific binding between the second ligand (L2) in the conjugate (L2-M) and the biological substance (O);

iv) Assaying the biological substance (O) by assaying the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-L2-M) .

36. An analytical method comprising the following elements i) to iv):

i) Using the analytical kit according to Claim 5;

ii) Introducing two or more of the materials a, b and c given below, either after preliminary mixing for conjugate formation or while allowing conjugate formation, into the passage in the analytical device contained in the analytical kit, followed by introduction of the remaining material, if any, into the passage:

a. A liquid sample suspected of the occurrence therein of a biological substance (O) to be assayed,

b. A reagent B' containing a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed,

c. A reagent C containing a conjugate (L3-M) composed of a third ligand (L3) capable of specifically binding to the second ligand (L2) and a marker (M);

iii) Allowing the formation of an immobilized conjugate (N1-N2-L1-O-L2-L3-M) by specific binding between the first ligand (L1) in the conjugate (N1-N2-L1) immobilized in the capturing zone in the analytical device and the biological substance (O), specific binding between the second ligand (L2) and biological substance (O) and specific binding between the second ligand and third ligand;

iv) Assaying the biological substance (O) by assaying the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-L2-L3-M).

37. An analytical method comprising the following elements

i) to iv):

i) Using the analytical kit according to Claim 5;

ii) Introducing the following materials a, b and c individually, without mixing together, into the passage in the analytical device contained in the analytical kit:

a. A liquid sample suspected of the occurrence therein of a biological substance (O) to be assayed,

b. A reagent B' containing a second ligand (L2) capable of specifically binding to the biological substance (O) to be assayed,

c. A reagent C containing a conjugate (L3-M) composed of a third ligand (L3) capable of specifically binding to the second ligand (L2) and a marker (M);

iii) Allowing the formation of an immobilized conjugate (N1-N2-L1-O-L2-L3-M) by specific binding between the first ligand (L1) in the conjugate (N1-N2-L1) immobilized in the capturing zone in the analytical device and the biological substance (O), specific binding between the second ligand (L2) and biological substance (O) and specific binding between the second ligand and third ligand;

iv) Assaying the biological substance (O) by assaying the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-L2-L3-M).

38. An analytical method comprising the following elements i) to iv):

i) Using the analytical kit according to Claim 6;

ii) Introducing two or more of the materials a, b and c specified below, either after mixing together for conjugate formation or

while allowing conjugate formation, into the passage in the analytical device contained in the analytical kit, followed by further introduction of the remaining material, if any, into the passage:

a. A liquid sample suspected of the occurrence therein of one or more biological substance species ( $Ok$ :  $k$  being an integer) to be assayed,

b. A reagent A solution containing conjugate species ( $N2h-L1i$ :  $h$  and  $i$  each independently being an integer) each composed of one of a plurality of second nucleic acid species ( $N2h$ :  $h$  being an integer), which has a base sequence at least complementary to the corresponding species among the plurality of first nucleic acid species ( $N1g$ :  $g$  being an integer) immobilized each independently, from species to species, in the capturing zone, and one of a plurality of first ligand species ( $L1i$ :  $i$  being an integer), which is capable of specifically binding to the corresponding species among the one or more biological substance species to be assayed;

c. A reagent B containing conjugate species ( $L2j-M1$ :  $j$  and  $l$  each independently being an integer) each composed of one of one or more second ligand species ( $L2j$ :  $j$  being an integer), which is capable of specifically binding to the corresponding species among the biological substance species ( $Ok$ :  $k$  being an integer), and one of one or more marker species ( $M1$ :  $l$  being an integer);

iii) Allowing the formation of conjugate species ( $N1g-N2h-L1i-Ok-L2j-M1$ :  $g$ ,  $h$ ,  $i$ ,  $j$ ,  $k$  and  $l$  each independently

being an integer) immobilized each independently, from species to species, by specific binding between the plurality of first nucleic acid species ( $N1g$ :  $g$  being an integer) immobilized independently, from species to species, in the capturing zone in the analytical device and the plurality of second nucleic acid species ( $N2h$ :  $h$  being an integer), specific binding between the plurality of first ligand species ( $L1i$ :  $i$  being an integer) and the one or more biological substance species ( $Ok$ :  $k$  being an integer) and specific binding between the one or more second ligand species ( $L2j$ :  $j$  being an integer) and the one or more biological substance species ( $Ok$ :  $k$  being an integer);

iv) assaying the one or more biological substance species ( $Ok$ :  $k$  being an integer) by assaying the one or more marker species ( $M1$ :  $l$  being an integer) contained in the plurality of immobilized conjugate species ( $N1g-N2h-L1i-Ok-L2j-M1$ :  $g, h, i, j, k$  and  $l$  each independently being an integer) obtained in the above step.

39. An analytical method comprising the following elements i) to iv):

i) Using the analytical kit according to Claim 6;  
ii) Introducing the following materials  $a, b$  and  $c$  individually, without mixing together, into the passage in the analytical device contained in the analytical kit:

a. A liquid sample suspected of the occurrence therein of one or more biological substance species ( $Ok$ :  $k$  being an integer) to be assayed,

b. A reagent A solution containing conjugate species

(N2h-L1i: h and i each independently being an integer) each composed of one of a plurality of second nucleic acid species (N2h: h being an integer), which has a base sequence at least complementary to the corresponding species among the plurality of first nucleic acid species (N1g: g being an integer) immobilized in the capturing zone, and one of a plurality of first ligand species (L1i: i being an integer), which is capable of specifically binding to the corresponding species among the one or more biological substance species to be assayed;

c. A reagent B containing conjugate species (L2j-M1: j and l each independently being an integer) each composed of one of one or more second ligand species (L2j: j being an integer), which is capable of specifically binding to the corresponding species among the biological substance species (Ok: k being an integer), and one of one or more marker species (M1: l being an integer);

iii) Allowing the formation of conjugate species (N1g-N2h-L1i-Ok-L2j-M1: g, h, i, j, k and l each independently being an integer) immobilized each independently, from species to species, by specific binding between the plurality of first nucleic acid species (N1g: g being an integer) immobilized independently, from species to species, in the capturing zone in the analytical device and the plurality of second nucleic acid species (N2h: h being an integer), specific binding between the plurality of first ligand species (L1i: i being an integer) and the one or more biological substance species (Ok: k being an integer) and specific binding between the one or more second



ligand species (L2j: j being an integer) and the one or more biological substance species (Ok: k being an integer);

iv) assaying the one or more biological substance species (Ok: k being an integer) by assaying the one or more marker species (Ml: l being an integer) contained in the plurality of immobilized conjugate species (N1g-N2h-L1i-Ok-L2j-Ml: g, h, i, j, k and l each independently being an integer) obtained in the above step.

40. An analytical method comprising the following elements i) to iv):

i) Using the analytical kit according to Claim 7;

ii) Introducing a mixture of two or more of the materials a, b, c and d given below as prepared in advance into the passage in the analytical device contained in the analytical kit, followed by introduction of the remaining material(s), if any, into the passage:

a. A liquid sample suspected of the occurrence therein of one or more biological substance species (Ok: k being an integer) to be assayed,

b. A reagent A solution containing conjugate species (N2h-L1i: h and i each independently being an integer) each composed of one of a plurality of second nucleic acid species (N2h: h being an integer), which has a base sequence at least complementary to the corresponding species among the plurality of first nucleic acid species (N1g: g being an integer) immobilized each independently, from species to species, in the capturing zone, and one of a plurality of first ligand species

(L1i: i being an integer), which is capable of specifically binding to the corresponding species among the one or more biological substance species to be assayed,

c. A reagent B' containing one or more second ligand species (L2j: j being an integer) each capable of specifically binding to the corresponding one among the one or more biological substance species (Ok: k being an integer) to be assayed, and

d. A reagent C containing conjugate species (L3m-M1: m and l each independently being an integer) each composed of one of one or more third ligand species (L3m: m being an integer), which is capable of specifically binding to the corresponding species among the second ligand species (L2j: j being an integer), and one of one or more marker species (M1: l being an integer);

iii) Allowing the formation of conjugate species (N1g-N2h-L1i-Ok-L2j-L3m-M1: g, h, i, j, k, l and m each independently being an integer) immobilized each independently, from species to species, by specific binding between the plurality of first nucleic acid species (N1g: g being an integer) immobilized independently, from species to species, in the capturing zone in the analytical device and the plurality of second nucleic acid species (N2h: h being an integer), specific binding between the plurality of first ligand species (L1i: i being an integer) and the one or more biological substance species (Ok: k being an integer), specific binding between the one or more second ligand species (L2j: j being an integer) and the one or more biological substance species (Ok: k being an integer) and specific binding between the one or more second ligand species

(L2j: j being an integer) and the one or more third ligand species (L3m: m being an integer);

iv) assaying the one or more biological substance species (Ok: k being an integer) by assaying the one or more marker species (Ml: l being an integer) contained in the plurality of immobilized conjugate species (N1g-N2h-L1i-Ok-L2j-L3m-Ml: g, h, i, j, k, l and m each independently being an integer).

41. An analytical method comprising the following elements i) to iv):

i) Using the analytical kit according to Claim 7;  
ii) Introducing the following materials a, b, c and d individually, without mixing together, into the passage in the analytical device contained in the analytical kit:

a. A liquid sample suspected of the occurrence therein of one or more biological substance species (Ok: k being an integer) to be assayed,

b. A reagent A solution containing conjugate species (N2h-L1i: h and i each independently being an integer) each composed of one of a plurality of second nucleic acid species (N2h: h being an integer) having a base sequence at least complementary to the corresponding species among the plurality of first nucleic acid species (N1g: g being an integer) immobilized each independently, from species to species, in the capturing zone, and one of a plurality of first ligand species (L1i: i being an integer), which is capable of specifically binding to the corresponding species among the one or more

biological substance species to be assayed,

c. A reagent B' containing one or more second ligand species (L2j: j being an integer) each capable of specifically binding to the corresponding one among the one or more biological substance species (Ok: k being an integer) to be assayed, and

d. A reagent C containing conjugate species (L3m-Ml: m and l each independently being an integer) each composed of one of one or more third ligand species (L3m: m being an integer), which is capable of specifically binding to the corresponding species among the second ligand species (L2j: j being an integer), and one of one or more marker species (Ml: l being an integer);

iii) Allowing the formation of conjugate species (N1g-N2h-L1i-Ok-L2j-L3m-Ml: g, h, i, j, k, l and m each independently being an integer) immobilized each independently, from species to species, by specific binding between the plurality of first nucleic acid species (N1g: g being an integer) immobilized each independently, from species to species, in the capturing zone in the analytical device and the plurality of second nucleic acid species (N2h: h being an integer), specific binding between the plurality of first ligand species (L1i: i being an integer) and the one or more biological substance species (Ok: k being an integer), specific binding between the one or more second ligand species (L2j: j being an integer) and the one or more biological substance species (Ok: k being an integer) and specific binding between the one or more second ligand species (L2j: j being an integer) and the one or more third ligand species (L3m: m being an integer);

iv) assaying the one or more biological substance species (Ok: k being an integer) by assaying the one or more marker species (Ml: l being an integer) contained in the plurality of immobilized conjugate species (Nlg-N2h-Lli-Ok-L2j-L3m-Ml: g, h, i, j, k, l and m each independently being an integer).

42. An analytical method comprising the following elements i) to v):

- i) Using the analytical kit according to Claim 8;
- ii) Preparing in advance one or more marker-carrying biological substance species (Ok-Ml: k and l each independently being an integer) from a liquid sample suspected of the occurrence therein of one or more biological substance species (Ok: k being an integer) by introduction of one or more marker species (Ml: l being an integer) into those biological substance species;
- iii) Introducing a reagent A containing conjugate species (N2h-Lli: h and i each independently being an integer) each composed of one of a plurality of second nucleic acid species (N2h: h being an integer), which has a base sequence at least complementary to the corresponding species among the plurality of first nucleic acid species (Nlg: g being an integer) immobilized each independently in a capturing zone, and one of a plurality of first ligand species (Lli: i being an integer) capable of specifically binding to the one or more biological substance species (Ok: k being an integer) and the one or more marker-carrying biological substance species, either after mixing together for conjugate formation or while allowing

conjugate formation, into the passage in the analytical device contained in the analytical kit;

iv) Allowing the formation of conjugate species (N1g-N2h-L1i-Ok-M1: g, h, i, k and l each independently being an integer), immobilized each independently, by specific binding between the plurality of first nucleic acid species (N1g: g being an integer) immobilized each independently in the capturing zone and the plurality of second nucleic acid species (N2h: h being an integer) and specific binding between the plurality of first ligand species (L1i: i being an integer) and the one or more biological substance species (Ok: k being an integer);

v) Assaying the one or more biological substance species (Ok: k being an integer) by assaying the one or more marker species (M1: l being an integer) contained in the plurality of immobilized conjugate species (N1g-N2h-L1i-Ok-M1: g, h, i, j, k and l each independently being an integer).

43. An analytical method comprising the following elements i) to v):

i) Using the kit according to Claim 8;

ii) Preparing in advance one or more marker-carrying biological substance species (Ok-M1: k and l each independently being an integer) from a liquid sample suspected of the occurrence therein of one or more biological substance species (Ok: k being an integer) by introduction of one or more marker species (M1: l being an integer) into those biological substance species;

iii) Introducing a reagent A containing conjugate species

(N2h-L1i: h and i each independently being an integer) each composed of one of a plurality of second nucleic acid species (N2h: h being an integer), which has a base sequence at least complementary to the corresponding species among the plurality of first nucleic acid species (N1g: g being an integer) immobilized each independently in a capturing zone, and one of a plurality of first ligand species (L1i: i being an integer) capable of specifically binding to the one or more biological substance species (Ok: k being an integer) and the one or more marker-carrying biological substance species, individually without mixing together, into the passage in the analytical device contained in the analytical kit;

iv) Allowing the formation of conjugate species (N1g-N2h-L1i-Ok-M1: g, h, i, k and l each independently being an integer), immobilized each independently, by specific binding between the plurality of first nucleic acid species (N1g: g being an integer) immobilized each independently in the capturing zone and the plurality of second nucleic acid species (N2h: h being an integer) and specific binding between the plurality of first ligand species (L1i: i being an integer) and the one or more biological substance species (Ok: k being an integer);

v) Assaying the one or more biological substance species (Ok: k being an integer) by assaying the one or more marker species (M1: l being an integer) contained in the plurality of immobilized conjugate species (N1g-N2h-L1i-Ok-M1: g, h, i, j, k and l each independently being an integer).

44. An analytical method comprising the following elements i) to iv):

i) Using the analytical kit according to Claim 9;

ii) Introducing the materials a and b specified below, either after mixing together for conjugate formation or while allowing conjugate formation, into the passage in the analytical device contained in the analytical kit:

a. A liquid sample suspected of the occurrence therein of one or more biological substance species (Ok: k being an integer),

b. A reagent containing conjugate species (L2j-Ml: j and l each independently being an integer) resulting from direct binding between one or more second ligand species (L2j: j being an integer) capable of specifically binding to the corresponding species among the one or more biological substance species (Ok: k being an integer) and one or more marker species (Ml: l being an integer);

iii) Allowing the formation of conjugate species (N1g-N2h-L1i-Ok-L2j-Ml: g, h, i, j, k and l each independently being an integer), immobilized each independently, by specific binding between the plurality of first ligand species (L1i: i being an integer) in the conjugate species (N1g-N2h-L1i: g, h and i each independently being an integer) immobilized each independently, from species to species, in the capturing zone in the analytical device and the one or more biological substance species (Ok: k being an integer) and specific binding between the one or more second ligand species (L2j: j being an integer)



in the conjugate species (L2j-Ml: j and l each independently being an integer) in the reagent and the one or more biological substance species (Ok: k being an integer);

iv) Assaying the one or more biological substance species (Ok: k being an integer) by assaying the one or more marker species (Ml: l being an integer) contained in the plurality of immobilized conjugate species (N1g-N2h-L1i-Ok-L2j-Ml: g, h, i, j, k and l each independently being an integer).

45. An analytical method comprising the following elements i) to iv):

i) Using the analytical kit according to Claim 9;  
ii) Introducing the following materials a and b individually, without mixing together, into the passage in the analytical device contained in the analytical kit:

a. A liquid sample suspected of the occurrence therein of one or more biological substance species (Ok: k being an integer),

b. A reagent containing conjugate species (L2j-Ml: j and l each independently being an integer) resulting from binding between one or more second ligand species (L2j: j being an integer) capable of specifically binding to the corresponding species among the one or more biological substance species (Ok: k being an integer) and one or more marker species (Ml: l being an integer);  
iii) Allowing the formation of conjugate species (N1g-N2h-L1i-Ok-L2j-Ml: g, h, i, j, k and l each independently being an integer), immobilized each independently, by specific

binding between the plurality of first ligand species ( $L1i$ :  $i$  being an integer) in the conjugate species ( $N1g-N2h-L1i$ :  $g$ ,  $h$  and  $i$  each independently being an integer) immobilized each independently, from species to species, in the capturing zone in the analytical device and the one or more biological substance species ( $Ok$ :  $k$  being an integer) and specific binding between the one or more second ligand species ( $L2j$ :  $j$  being an integer) in the conjugate species ( $L2j-M1$ :  $j$  and  $l$  each independently being an integer) in the reagent and the one or more biological substance species ( $Ok$ :  $k$  being an integer);

iv) Assaying the one or more biological substance species ( $Ok$ :  $k$  being an integer) by assaying the one or more marker species ( $M1$ :  $l$  being an integer) contained in the plurality of immobilized conjugate species ( $N1g-N2h-L1i-Ok-L2j-M1$ :  $g$ ,  $h$ ,  $i$ ,  $j$ ,  $k$  and  $l$  each independently being an integer).

46. An analytical method comprising the following elements i) to iv):

i) Using the analytical kit according to Claim 10;

ii) Introducing two or more of the materials  $a$ ,  $b$  and  $c$  specified below, either after mixing together in advance for conjugate formation or while allowing conjugate formation, into the passage in the analytical device contained in the analytical kit, followed by introduction of the remaining material, if any, into the passage:

a. A liquid sample suspected of the occurrence therein of one or more biological substance species ( $Ok$ :  $k$  being an

integer) to be assayed,

b. A reagent B' containing one or more second ligand species (L2j: j being an integer) each capable of specifically binding to the corresponding one among the one or more biological substance species (Ok: k being an integer) to be assayed,

c. A reagent C containing conjugate species (L3m-Ml: m and l each independently being an integer) each composed of one of one or more third ligand species (L3m: m being an integer), which is capable of specifically binding to the corresponding species among the second ligand species (L2j: j being an integer), and one of one or more marker species (Ml: l being an integer);

iii) Allowing the formation of immobilized conjugate species (N1g-N2h-L1i-Ok-L2j-L3m-Ml: g, h, i, j, k, l and m each independently being an integer) by specific binding between the first ligand species (L1i: i being an integer) in the conjugate species (N1g-N2h-Mli: g, h and i each independently being an integer) immobilized each independently in the capturing zone in the analytical device and the biological substance species (Ok: k being an integer), specific binding between the second ligand species (L2j: j being an integer) and the biological substance species (Ok: k being an integer) and specific binding between the second ligand species (L2j: j being an integer) and the third ligand species (L3m: m being an integer);

iv) Assaying the one or more biological substance species (Ok: k being an integer) by assaying the one or more marker species (Ml: l being an integer) contained in the immobilized conjugate species (N1g-N2h-L1i-Ok-L2j-L3m-Ml: g, h, i, j, k, l and m each

independently being an integer).

47. An analytical method comprising the following elements i) to iv):

i) Using the analytical kit according to Claim 10;  
ii) Introducing the following materials a, b and c individually, without mixing together, into the passage in the analytical device contained in the analytical kit:

a. A liquid sample suspected of the occurrence therein of one or more biological substance species ( $Ok$ :  $k$  being an integer) to be assayed,

b. A reagent  $B'$  containing one or more second ligand species ( $L2j$ :  $j$  being an integer) each capable of specifically binding to the corresponding one among the one or more biological substance species ( $Ok$ :  $k$  being an integer) to be assayed,

c. A reagent C containing conjugate species ( $L3m-Ml$ :  $m$  and  $l$  each independently being an integer) each composed of one of one or more third ligand species ( $L3m$ :  $m$  being an integer), which is capable of specifically binding to the corresponding species among the second ligand species ( $L2j$ :  $j$  being an integer), and one of one or more marker species ( $Ml$ :  $l$  being an integer);

iii) Allowing the formation of immobilized conjugate species ( $N1g-N2h-L1i-Ok-L2j-L3m-Ml$ :  $g$ ,  $h$ ,  $i$ ,  $j$ ,  $k$ ,  $l$  and  $m$  each independently being an integer) by specific binding between the first ligand species ( $L1i$ :  $i$  being an integer) in the conjugate species ( $N1g-N2h-Mli$ :  $g$ ,  $h$  and  $i$  each independently being an integer) immobilized each independently in the capturing zone

in the analytical device and the biological substance species (Ok: k being an integer), specific binding between the second ligand species (L2j: j being an integer) and the biological substance species (Ok: k being an integer) and specific binding between the second ligand species (L2j: j being an integer) and the third ligand species (L3m: m being an integer);

iv) Assaying the one or more biological substance species (Ok: k being an integer) by assaying the one or more marker species (Ml: l being an integer) contained in the immobilized conjugate species (N1g-N2h-L1i-Ok-L2j-L3m-Ml: g, h, i, j, k, l and m each independently being an integer).

48. An analytical method comprising the following elements i) to v):

- i) Using the analytical device according to Claim 18;
- ii) Preparing in advance a marker-carrying biological substance (O-M) from a liquid sample suspected of the occurrence therein of a biological substance (O) by introduction of a marker (M) thereinto;
- iii) Introducing the marker-carrying biological substance (O-M) into the passage in the analytical device;
- iv) Allowing the formation of an immobilized conjugate (N1-N2-L1-O-M) by specific binding between the first ligand (L1) in the conjugate (L1-N2) composed of the first ligand (L1) and second nucleic acid (N2) and immobilized in the capturing zone in the analytical device and the biological substance (O) in the marker-carrying biological substance (O-M);

v) Assaying the biological substance (O) by assaying the marker (M) contained in the immobilized conjugate (N1-N2-L1-O-M).

49. An analytical method comprising the following elements i) to v):

- i) Using the analytical device according to Claim 19;
- ii) Preparing in advance one or more marker-carrying biological substance species (Ok-Ml: k and l each independently being an integer) from a liquid sample suspected of the occurrence therein of one or more biological substance species (Ok: k being an integer) by introduction of one or more marker (Ml: l being an integer) thereinto;
- iii) Introducing the marker-carrying biological substance species (Ok-Ml: k and l each independently being an integer) into the passage in the analytical device;
- iv) Allowing the formation of immobilized conjugate species (N1g-N2h-L1i-Ok-Ml: g, h, i, k and l each independently being an integer) by specific binding between the plurality of first ligand species (L1i; i being an integer) immobilized each independently in the capturing zone in the analytical device and the one or more biological substance species (Ok: k being an integer) in the one or more marker-carrying biological substance species (Ok-Ml: k and l each independently being an integer);
- v) Assaying the one or more biological substance species (Ok: k being an integer) by assaying the one or more marker species

(M1: 1 being an integer) contained in the immobilized conjugate species (N1g-N2h-L1i-Ok-M1: g, h, i, k and l each independently being an integer).

50. An analytical method according to any of Claims 24 to 49, wherein the rate of flow through the passage is 0.1 to 50  $\mu$ L/minute.

51. A method of preparing analytical devices which is characterized by:

(1) Preparing a first member having a groove, 1  $\mu$ m to 5 mm width and 1  $\mu$ m to 750  $\mu$ m depth in cross-section, and a second member capable of covering the groove,

wherein the groove is a portion to become a passage upon joining the first member and second member together and one of the first member and second member or both have a passage inlet and a passage outlet,

(2) Immobilizing a nucleic acid (N) having an arbitrary base sequence at a site to become a zone for capturing a biological substance to be assayed in a portion to become a passage on the first member and/or second member,

(3) Then, joining the first member and second member together by thermal fusion or with an adhesive to give an assembly with a passage formed therein,

(4) Introducing a reagent A containing a conjugate (N2-L1) composed of a second nucleic acid (N2) having a base sequence at least complementary to the base sequence of the first nucleic acid (N1) immobilized in the capturing zone and a first ligand

(L1) capable of specifically binding to a biological substance to be assayed into the passage in the assembly, and allowing the conjugate (N2-L1) to specifically bind, for immobilization thereof, to the first nucleic acid (N1) in the capturing zone.

52. A method of preparing analytical devices which is characterized by:

(1) Preparing a first member having a groove, 1  $\mu\text{m}$  to 5 mm width and 1  $\mu\text{m}$  to 750  $\mu\text{m}$  depth, and a second member capable of covering the groove,

wherein the groove is a portion to become a passage upon joining the first member and second member together and one of the first member and second member or both have a passage inlet and a passage outlet,

(2) Immobilizing a plurality of nucleic acid species (N1g: g being an integer) each having an arbitrary base sequence, each independently, at a site to become a zone for capturing one or more biological substance species to be assayed within a portion to become a passage on the first member and/or second member,

(3) Then, joining the first member and second member together by thermal fusion or with an adhesive to give an assembly with a passage formed therein,

(4) Introducing a reagent A containing conjugate species (N2h-L1i: h and i each independently being an integer) each composed of one of a plurality of second nucleic acid species (N2h: h being an integer), which has a base sequence at least complementary to the base sequence of the corresponding species



among the plurality of first nucleic acid species ( $N1g$ :  $g$  being an integer) immobilized in the capturing zone, and one of a plurality of first ligand species ( $L1i$ :  $i$  being an integer), which is capable of specifically binding to the corresponding species among one or more biological substance species to be assayed into the passage in the assembly, and allowing the plurality of conjugate species ( $N2h-L1i$ :  $h$  and  $i$  each independently being an integer) to specifically bind, for immobilization thereof, to the plurality of first nucleic acid species ( $N1g$ :  $g$  being an integer) in the capturing zone.

53. A method of preparing analytical devices as set forth in Claim 51 or 52, wherein the fusion temperature is  $70^{\circ}\text{C}$  to  $140^{\circ}\text{C}$ .

54. A method of preparing analytical devices as set forth in Claim 51 or 52, wherein the biological substance or substances and/or first ligand ( $L1$ ) or ligands are selected from among immunological substances, receptors and nucleic acids.

55. A method of preparing analytical devices as set forth in Claim 51 or 52, wherein the first member or second member material is selected from among glass, polydimethylsiloxane, ceramics, acrylonitrile-butadiene rubber-styrene resins, acrylonitrile-ethylene propylene rubber-styrene resins, acrylonitrile-styrene resins, methacrylic-styrene resins, polyamide nylon resins, polybutylene terephthalate resins, polycarbonate resins, polyethylene resins, polyethylene resins,

polyethylene terephthalate polyester resins, polyimide resins, methacrylic resins, polyacetal resins, polypropylene resins, polyphenylene ether resins, polyphenylene sulfide resins, polystyrene resins, thermoplastic elastomer resins, alloys, liquid crystal polymer resins, cycloolefin resins, thermoplastic resins, epoxy resins, phenol resins, unsaturated polyester resins, diallyl phthalate resins, cyclic olefin copolymers and, further, materials derived from these materials by surface modification.

56. A method of preparing analytical devices as set forth in Claim 51 or 52, wherein the first member and second member are made of the same material.

57. A method of preparing analytical devices as set forth in Claim 51 or 52, wherein the material of the first member and the material of the second member are different from each other.